

## **Amendments to the specification**

Please replace the paragraph beginning on page 2, line 21 with the following paragraph:

A large and varied class of restriction endonucleases has been classified as 'Type II' class of restriction endonucleases. These enzymes cleave DNA at defined positions, and when purified can be used to cut DNA molecules into precise fragments for gene cloning and analysis. The biochemical precision of Type II restriction endonucleases far exceeds anything achievable by chemical methods, making these enzymes the reagents *sine qua non* of molecular biology laboratories. In this capacity as molecular tools for gene dissection Type II restriction endonucleases have had a profound impact on the life sciences and medicine in the past 25 years, transforming the academic and commercial arenas, alike. Their utility has spurred a continuous search for new restriction endonucleases, and a large number have been found: today more than 250 Type II endonucleases are known, each possessing different DNA cleavage characteristics (Roberts, R.J. et al., *Nucl. Acids. Res.* 33:D230-D232 (2005)). (Rebase, <http://rebase.neb.com/rebase>). The production and purification of these enzymes have also been improved by the cloning and overexpression of the genes that encode them, usually in the context of non-native host cells such as *E. coli*.

Please replace the paragraph beginning on page 14, line 5 with the following paragraph:

For any unknown restriction endonuclease that is observed to have a modular structure, the recognition sequence of the endonuclease of the class may be determined by mapping the locations of the cleavage sites in a target DNA of known sequence. The DNA sequences of these regions are compared for similarity and common features. Candidate recognition sequences are compared with the observed restriction fragments produced by endonuclease-cleavage of a variety of DNAs. The approximate size of DNA fragments produced by endonuclease digestion can be entered into the program REBPredictor, which can be accessed at <http://taq.neb.com/~vincze/REBpredictor/index.php>. Example III describes how REBPredictor was used to predict potential recognition sites for CspCI.

Please replace the paragraph beginning on page 41, line 8 with the following paragraph:

The endonuclease CspCI was found to cleave PhiX174 DNA twice, producing fragments of approximately 3300 bp and 2050 bp. The locations of the cut sites were mapped to approximate positions of nt 1575 and nt 4875 by simultaneously digesting PhiX174 DNA with CspCI and with additional restriction endonucleases which cleave at known positions, such as PstI, SspI, NciI, and StuI (Figure 1). CspCI did not cut pBR322 DNA or pUC19 DNA. The approximate size of the DNA fragments produced by CspCI digestion of phage lambda DNA (18 kb, 11 kb, 8.3 kb, 5.1 kb, 4.3 kb and 1.8 kb) were entered into the program REBPredictor, which can be accessed at <http://taq.neb.com/~vincze/REBpredictor/index.php>.

Please amend the paragraph beginning on page 14, line 9 as follows:

A modular endonuclease of the type described above can be obtained as a product of recombination in a host cell or by culturing the native strain. Host cells are grown in suitable media supplemented with 100  $\mu\text{mg/ml}$  ampicillin and incubated aerobically at 37°C. Cells in the late logarithmic stage of growth are collected by centrifugation and either disrupted immediately or stored frozen at -70°C.

Please amend the paragraph beginning on page 17, line 15 as follows:

The diluted enzyme was applied to a 375  $\mu\text{ml}$  Heparin Hyper-D column (Biosepra, Marlborough MA), which had been equilibrated in buffer B. (20mM Tris-HCl (pH 7.4), 150mM NaCl, 1.0mM DTT, 0.1mM EDTA, 5% Glycerol). A 2.5 L wash of buffer B was applied, then a 2 L gradient of NaCl from 0.15M to 1M in buffer B was applied and fractions were collected. Fractions were assayed for CspCI endonuclease activity by incubating with 1 microgram of phage lambda DNA (~~NEB~~ New England Biolabs, Inc., Ipswich, MA) in 50 microliter NEBuffer 2, supplemented with 20 microMolar (AdoMet) for 15 minutes at 37°C. CspCI activity eluted at 0.3M to 0.35M NaCl.

Please amend the paragraph beginning on page 20, line 1 as follows:

AdoMet: Supplementing the CspCI reaction with 20  $\mu\text{M}$  AdoMet greatly enhanced the activity of the enzyme. In reactions where AdoMet was omitted, the enzyme exhibited less than 5% of the cutting activity it exhibited in the AdoMet-supplemented reactions, indicating that AdoMet is a necessary cofactor for this enzyme.

Please amend the paragraph beginning on page 20, line 15 as follows:

Digestion at 37°C for one hour in the following NEBuffers yielded the following approximate percentage cleavage activities relative to NEBuffer 2 (New England Biolabs, Inc, Beverly Ipswich, MA)+ 20µmM AdoMet:

NEBuffer 1 + 20µmM AdoMet: 10%  
NEBuffer 2 + 20µmM AdoMet: 100%  
NEBuffer 3 + 20µmM AdoMet: 10%  
20 NEBuffer 4 + 20µmM AdoMet: 75%  
NEBuffer 2 - (No AdoMet): < 5%

Please amend the paragraph beginning on page 22, line 24 as follows:

The sequencing reactions were performed using the Sequenase version 2.0 DNA sequencing kit (GE Healthcare, formerly Amersham Life Science) with modifications for the cleavage site determination. The template and primer were assembled in a 0.5 ml Eppendorf tube by combining 2.5 microliter dH<sub>2</sub>O, 3 microliter 5X sequencing buffer (200 mM Tris pH 7.5, 250 mM NaCl, 100 mM MgCl<sub>2</sub>), 8 microliter M13mp18 single-stranded DNA (1.6 microgram) and 1.5 microliter of primer at 3.2 µmM concentration. The primer-template solutions were incubated at 65 °C for 2 minutes, then cooled to 37°C over 20 minutes in a beaker of 65°C water on the bench top to anneal the primer. The labeling mix (diluted 1:20) and T7 Sequenase polymerase were diluted according to manufacturer's instructions. The annealed primer and template tube was placed on ice. To this tube were added 1.5 microliter 100mM DTT, 3 microliter diluted dGTP labeling mix, 1 microliter [ $\alpha$ - <sup>33</sup>P] dATP (2000Ci/mM, 10mCi/ml) and 3 microliter diluted T7 Sequenase polymerase (GE Healthcare, formerly Amersham, Piscataway,

NJ). The reaction was mixed and incubated at room temperature for 4 minutes.

Please amend the paragraph beginning on page 24, line 5 as follows:

10 microliter of the extension reaction was then placed in zone 0.5 ml Eppendorf tube and 7 microliter was placed in a second tube. To the first tube was added 1 microliter (approximately 0.5 unit) of CspCI endonuclease, The reaction was mixed, and then 2 microliter was transferred to the second tube. These enzyme digest reactions were mixed and then incubated at 37°C for 1 hour, following which the reactions were divided in half. To one half, 4 microliter of stop solution was added and mixed (the 'minus' polymerase reaction). To the second half, 0.4 microliter Klenow DNA polymerase (NEB#210) (New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) containing 80  $\mu$ M dNTPs was added (the 'plus' reaction), and the reaction was incubated at room temperature for 15 minutes, following which 4 microliter of stop solution was added.

Please amend the paragraph beginning on page 29, line 3 as follows:

Unambiguous results were obtained for the positions of cleavage on the 5' sides of the recognition sequence, but the data was poorer regarding cleavage on the 3' sides. As a whole, however, they were consistent with the endonuclease cleaving to produce fragments with 2-base 3'-overhangs-~~at~~. Sequence traces from representative reactions are shown in Figure 5.